

## Comparison of salivary cortisol concentrations in Jaguars kept in captivity with differences in exposure to the public

### Comparação das concentrações de cortisol salivar em onças-pintadas criadas em cativeiro com diferenças de exposição ao público

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#### ABSTRACT

*For the most part, jaguars kept in captivity are used for educational and recreational purposes and it hasn't been determined the different impacts that public exposure would have on these animals. In this study, we compared the salivary cortisol, one of the stress indicator hormones, in seven captive jaguars exposed to the high and low public visitation. Saliva was collected using an absorbent material that was chewed by the animals and subsequently analyzed through an immunoenzymatic assay. Salivary cortisol concentrations were significantly higher in the jaguars with higher public exposure, which suggest a more hormonal stress response. These results indicated that jaguars, animals usually of solitary and secretive behavior, may have their welfare considerably diminished by increased public exposure. Additionally, design of the enclosures, without refuges and proximity of people, could predispose jaguars to psychological stress.*

**Key words:** *Panthera onca*, jaguar, salivary cortisol, welfare.

#### RESUMO

*Em geral, onças-pintadas criadas em cativeiro são usadas para fins educacionais e de recreação. No entanto, o impacto da exposição ao público sobre esses animais não tem sido avaliado. Neste estudo, foi comparado o impacto de dois diferentes níveis de exposição ao público em sete onças-pintadas, alojadas em dois criadouros, por meio da avaliação de um dos hormônios indicadores de estresse, o cortisol salivar. A saliva foi coletada usando um material absorvente que foi mastigado pelos animais e subsequentemente analisado por imunoenzimoensaio. As concentrações de cortisol salivar foram significativamente maiores em onças-pintadas com maior exposição ao público. Esses resultados sugerem que onças-pintadas, normalmente solitárias e esquivas na natureza,*

*podem ter seu bem-estar consideravelmente diminuído pelo aumento de exposição ao público. Este estudo é o primeiro relato de mensuração de cortisol salivar em onças-pintadas.*

**Palavras-chave:** *Panthera onca*, onça-pintada, cortisol salivar, bem-estar.

#### INTRODUCTION

The reprehension of institutions involved in fauna conservation has resulted several times in an exceeding accumulation of big felids, which have a difficult maintenance. There is an increased effort to set part of the jaguar (*Panthera onca*) breed kept in captivity to be reintroduced in its natural habitat (SANDERSON et al., 2002). However, some animals can not be reintroduced to nature due to long captivity periods, lesions incurred during their life, behavioral idiosyncrasies and contamination by urban pathogens that could be disseminated within the wild population (CUNNINGHAM, 1996). Although these captive animals can be used in environmental awareness programs and for recreational means in zoos, they are housed in the most diverse conditions. Another purpose, for both zoos and other raising facilities, is to foment databases of physiological data, such as the levels of salivary cortisol, as a reference to studies in captivity and in natural settings.

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The success of raising wild animals depends in great part on the conditions they are kept in. For jaguars, these conditions are far from being completely understood, even though some studies have suggested efficient models (JAGUAR SSP MANAGEMENT GROUP, 2007). It is known that these animals need space, shelter, refuge and environmental enrichment in order to satisfy their behavioral needs (JAGUAR SSP MANAGEMENT GROUP, 2007). Although jaguars are considered sensible to routine changes and noise, paradoxically they seem to ignore exposure to public (JAGUAR SSP MANAGEMENT GROUP, 2007). In practice, reports from animal caretakers and zoo staff describes a tendency of jaguars to be readjust to human presence as compared to lions and pumas, for example (Cristina Gianni, NEX). A solitary, territorial life, in great forest expanses usually associated to water courses (POLISAR et al., 2003), could account to the predisposition of jaguars to this readjustment.

Zoos are supposed to have public visitation, however, this may incur in disturbs to the animals routine (ROSS et al., 2005). In cases of environment perturbations to some felid species submitted to exposure to public, there can be signs of low welfare and stress (WIELEBNOWSKI, 2003). One parameter used to evaluate welfare and stress is the levels of corticosteroid hormones (cortisol or corticosterone). These hormones are generated by a cascade of biochemical events that originate in a primary stimulation of the limbic system, by emotional responses of defense and prolonged frustration (MORGAN & TROMBORG, 2007; TILBROOK & CLARKE, 2006). The final outcome is the stimulation of the hypothalamus-pituitary-adrenal (HPA) axis to increase the plasmic cortisol (TILBROOK & CLARKE, 2006). Animals in low stimuli, threatening and uncomfortable environments have high levels of corticosteroids (WIELEBNOWSKI, 2003; TILBROOK & CLARKE, 2006). High indices of plasmic, fecal, and salivary cortisol can be associated to psychological stress in several wild felids, as well, as domestic cats (NOGUEIRA & SILVA, 1997; CAUVIN et al., 2003; MORATO et al., 2004).

Due jaguars are particularly secretive towards human contact in their natural habitat (SCHALLER & CRAWSHAW Jr, 1980), this study tried to evaluate the impact in salivary cortisol levels of captive jaguars, as an indicative of stress and welfare, under high and moderate exposure to public.

## MATERIAL AND METHODS

Jaguars were evaluated in the Brasilia Zoo (BZ), Brasilia, and in the conservationist breeding facility "No Extinction" (NEX), in Pirenopolis, both in the Brazilian central highlands. The BZ group was composed of three jaguars: one 19 year old female (Juma), a 7 year old male (General), Juma's progeny, and another male of unknown origin, 8 years old (Tuan) approximately. Feeding was done with chunks of meat (bovine and chicken) at the end of the day, Mondays Wednesday and Fridays, at which time they are also removed to sleeping chambers, where they are kept until the next morning. The jaguar's keep is built of concrete, eleven meters deep, circular and with a 250m<sup>2</sup> area. It has two raised platforms (two meters high from the ground), an internal moat filed with water around the external perimeter of the keep, and an island in the center with shrubs. There were four gated subterranean rooms with access to the main area which were used for isolation and animals restraint. Zoo visitors have access to the keep on the top of the structure around the entirety of the perimeter, separated by metal bars which are distant one meter from the border of the main keep.

In the NEX, four jaguars were evaluated: three were kept together and one housed alone. The three animals kept together were composed by two females (Carlota and Dalila) and one male (Samson), all five years old and raised in captivity since birth. The remaining animal was an adult male (Gaviao), of unknown age, in captivity for at least six years. Gaviao was apprehended by Brazilian Institute of Environment officers in a farm where it was kept chained for "protection of the property". At the moment of apprehension it was noted that Gaviao had been de-clawed and castrated by the farmer. Animals at NEX are fed daily at the end of the day (bovine and equine meats, and poultry). The keep is set up as large enclosure surrounded by a thick metal mesh, containing in its interior trees, an artificial grotto to be used as refuge, a catwalk three meters high and an artificial pond of approximately 30m<sup>2</sup>. The ground is covered in grass. Access for visitors is made at the same level as the animals. Visitors can get as close as two meters from the keep and are further separated by a secondary metal screen that keeps out even small animals, such as birds and rodents.

All jaguars observed have no history of metabolic issues or any other major health problem. At time of sampling, jaguars were deemed in good health by a team of veterinarians.

The public visitation is higher in BZ than NEX. Except on Mondays, the public visitation is every day on a week, and it is highest at weekends with approximately two thousand people. The estimative of NEX public visitation is around 30 people at weekends (Saturdays and Sundays), and no visits happen at the rest of the week. The non-visit days for the BZ and for NEX jaguars were Mondays and Wednesday to Friday, respectively.

Salivary cortisol is a non-invasive reliable method to measure the cortisol profile related to environmental stimulus in wild animals in captivity (FUCHS et al., 1997; CROSS & ROGERS, 2004). The method of salivary cortisol collection used on pigs, primates and other animals was suited to jaguars. Saliva sampling was done using soft balls made of resistant, inert and absorbent cotton tissue to be chewed by the animals. The balls have approximately 15 cm of diameter, and are made of non-flavored cotton tissue and enroled with ties of resistant canvas. The sampling balls were thrown to the animals and the manipulation and chewing were observed. These were eventually retrieved and chewed on by the animals. After 30 minutes the ball removed from the enclosure had approximately 5 mL of saliva squeezed from them into assay tubes. Saliva was kept frozen at -20°C until laboratory analysis. This technique had been previously tested and the quality and quantity of the sample were deemed adequate for this analysis. Sampling was done from the months of March to May 2007, between the hours of 8 and 9 am, with the jaguars fasting for at least 24 hours. Samples from BZ were obtained on Mondays, Tuesdays and Thursdays, with three repetitions for each day of the week. Sampling at NEX was done from Wednesday to Monday, on a consecutive daily basis. On total, nine samples were taken from each jaguar located at BZ, and six from each jaguar at the NEX. Sampling was performed consistently and in the same order, always by the same researchers.

All cortisol measurements were made at the same time in the same assay, using an immunoenzymatic system (DSL10-67100/Active, USA) by Laboratorio Genese (Sao Paulo, Brazil). The laboratory used the regular procedure according to technical recommendations. In short, after thawing, the sample vials were centrifuged at 1000G for two minutes to produce a clear supernatant, which was aspirated with a micropipette (approximately 30 µL). Immediately the supernatant was dropped into tubes, shaken for 45 min and appropriately discarded. The plates were washed and incubated with a solution (TMB chromogen) for 15 min. Cortisol levels were determined by employing a

time-resolved enzymatic immunoassay, where cortisol in standards and unknowns competed for antibody binding sites. The bound cortisol was measured using an optical density microplate reader at 450 nm (Dynatech MR 5000, Dynatech Laboratories, USA).

A Mann-Whitney *U* test was used to determine the difference (if any) in the salivary cortisol levels between the two groups of jaguars. Samples were also classified according to visitation level (with and without visitation) at day of sampling and tested for differences within each group of jaguars.

The possibility of a linear relationship between salivary cortisol and the intensity of visitation were tested. The Spearman correlation test was used. Values used were the mean and standard error of sampling in each day for each animal; when necessary measurements from jaguars in each site were grouped. It was assumed alpha equals or smaller to 5% and a two-tailed distribution.

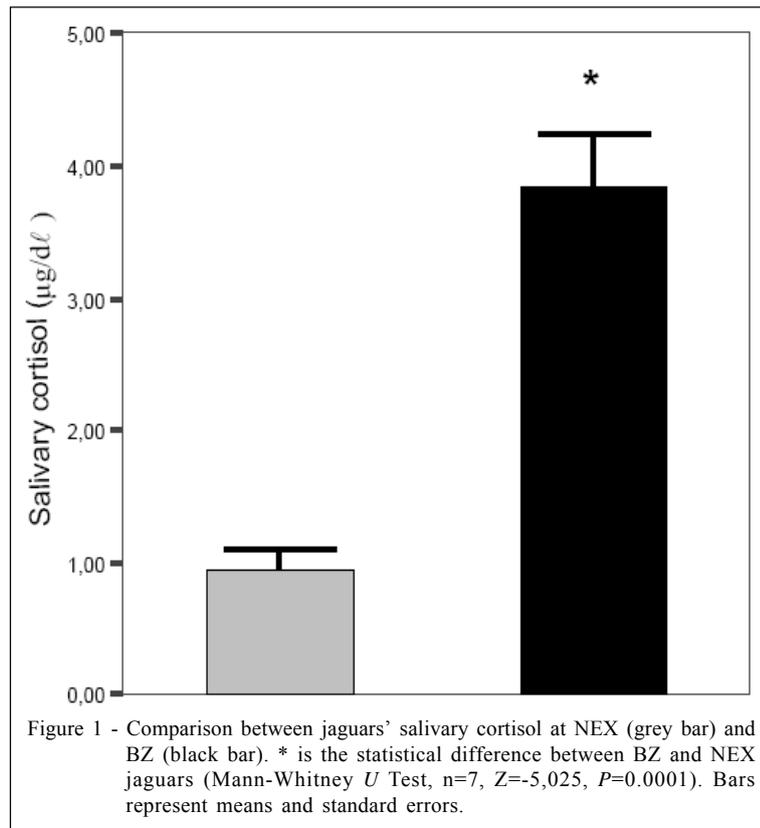
## RESULTS

There were no differences between the measured cortisol levels within each group of jaguars (BZ,  $P=0.49$ ; NEX,  $P=0.12$ ). When both groups of jaguars were compared to each other, BZ showed the highest salivary cortisol concentration (Figure 1). According to visitations per day, the BZ zoo showed the highest salivary cortisol concentrations in days of increased visitation (Figure 2). On jaguars of the NEX group there were no significant differences on salivary cortisol in days with different visitation intensity ( $P=0.22$ ).

There seemed to be a gradation on salivary cortisol levels in each day of the week, which prompted to test this using with a Spearman correlation test. There was a significant correlation between salivary cortisol levels and the day of the week it was sampled in for the BZ group ( $\rho=0.42$ ;  $P=0.01$ ), but not for the NEX group ( $\rho=0.05$ ;  $P=0.98$ ).

## DISCUSSION

The salivary cortisol level in jaguars from BZ group was higher than from jaguars of NEX group. Considering that the jaguars management, the age-sex class and genetic makeup are convergent for the NEX and BZ groups, precluding other sharp differences, the difference in salivary cortisol between each group of jaguars can be explained by the intensity of the visitation received, which was markedly different in each site studied. In several domestic species and wild mammals, salivary cortisol is correlated to plasmic



cortisol (domestic dog: BEERDA et al., 1996; guinea pig: FENSKE, 1996; rhesus monkey: DAVENPORT et al., 2003; dolphins: PEDERNERA-ROMANO et al., 2006) and its increasing is tied to distress, being consequence tied to the animal's welfare (LANE, 2006).

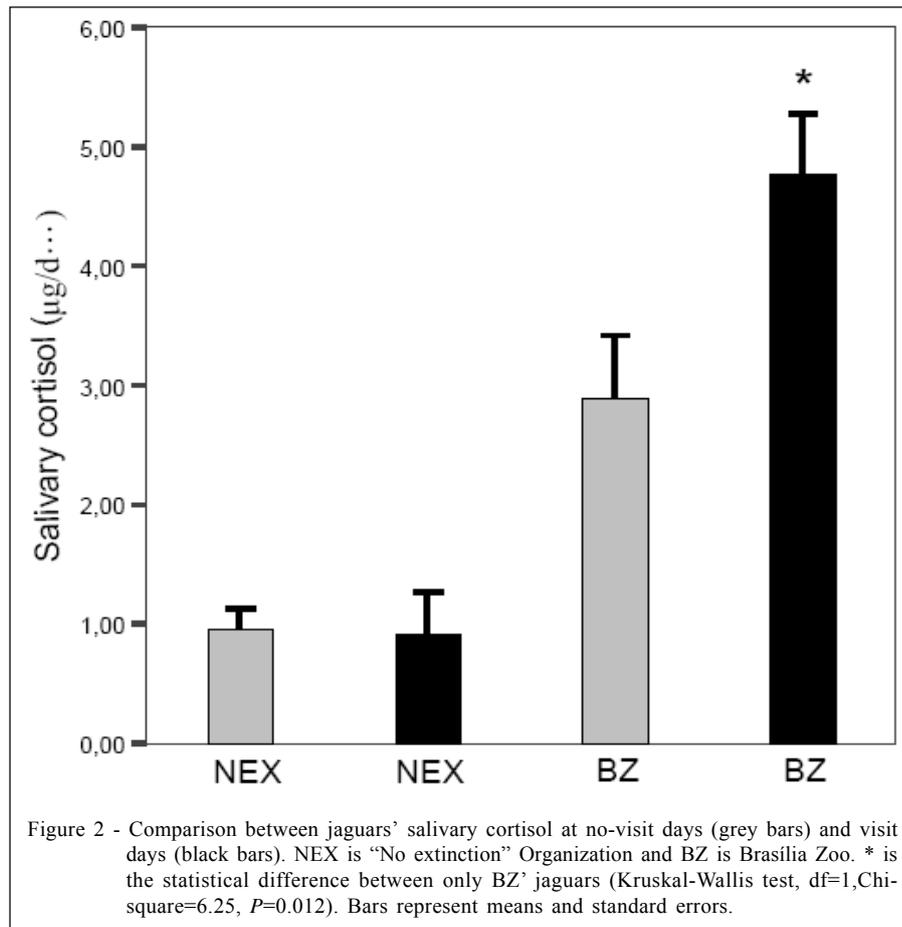
Natural behavioral characteristics tend to be preserved while the animal is in captivity (CLUBB & MASON, 2007). Frustration on performing the characteristic behavioral repertoire in the animals generates a neuron-endocrine predisposition to low levels of welfare. In its natural habitat, jaguars have a solitary and secretive behavior, being more active at dusk and over night, with predatory strategies of sudden attack (POLISAR et al., 2003). Exposure to intense visitation is directly opposite to what these animal are selected in their natural environment to do. Even when living in an ergonomically planned environment to house big felids, the increased levels of salivary cortisol in the BZ animals seems to indicate a lack of adaptation to captivity on these animals.

On the other hand, we can not discard the possibility of enclosures differences influencing the cortisol response. There is a plethora of studies showing different cortisol profile of animals in different enclosures designs (CLUBB & MASON, 2007). It was

suggested in this case, that the BZ jaguars place on the bottom of the enclosure is not suited to behavioral needs of jaguars. Most specifically, jaguar's refuges are on top of tress in nature (SCHALLER & CRAWSHAW, 1980). In the BZ enclosures, they are exposed on the bottom, without free access to refuges and surrounded by noisy visitors. In this case, the problem could not be the number of visitors but the place of them in relation to observed jaguars.

In comparison to the BZ facilities, visitors remain at the same level as the jaguars in the NEX, with a decreased safety area around the housing facilities, which allow a greater proximity of the public. Although having this seemingly higher exposure to the public, the jaguars in the NEX did not present higher salivary cortisol levels in periods of increased visitation. Also, cortisol levels were stably maintained during the week, without any indication of variation associated to any period in the week. By contrast, besides having significantly higher salivary cortisol levels on days with visitation, the animals in the BZ presented progressively increasing levels as the days of increased visitation approached.

On the BZ jaguars, the long captivity time and the increased exposure to the public could have



induced a habituation state, like several species maintained in captivity (VAN KRUNKELSVEN et al., 1999). The increased cortisol levels in the jaguars housed in the BZ facilities indicates that they have not habituated themselves with this increased human exposure. These results allow us to raise questions regarding the ideal number of visitors per day for jaguars kept in captivity, with an exact number yet to be determined, and still maintain their environment in reasonable level of well being. Alternatively, a more practical solution to this welfare issue is the adequate provision of protected parts of enclosure in which jaguars could escape from public exposure.

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#### BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The study was approved by the Animal Use Committee (CEUA) of the Brasilia University in line with IBAMA statement (Brazilian Institute of Environmental Protection). All procedures were not invasive and performed under veterinarian supervision.

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